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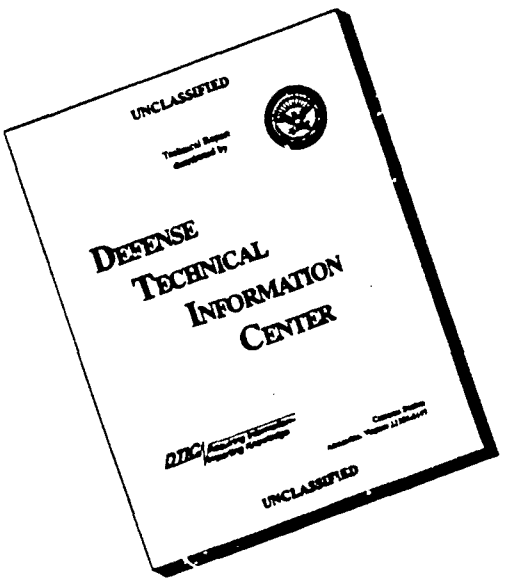

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SYNTHESIS OF NUCLEOSIDE MONO- AND DIALDEHYDES AS ANTIVIRAL AGENTS

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Annual Report

John P. Neenan, Ph.D.

15 December 1987

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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Rochester, New York 14623

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Two compounds, 4',5'-unsaturated adenosine-2',3'-dialdehyde and tubercidin-2',3'-dialdehyde were submitted during the reporting period. Work on the synthesis of adenosine-2'-monoaldehyde, adenosine-3'-monoaldehyde and 5'-deoxyadenosine-2',3'-dialdehyde is still in progress. As a group the nucleoside dialdehydes thus far submitted have shown broad spectrum activity against many of the viruses in the screening system, and some, like guanosine dialdehyde, have shown remarkably low cytotoxicity. Even dialdehydes that cannot become phosphorylated, which is required for the antiviral activity of all clinically used nucleoside antivirals, showed antiviral activity. For example 5'-fluoro-5'-deoxy-adenosine-2',3'-dialdehyde showed good activity against yellow fever and 4',5'-unsaturated adenosine-2',3'-dialdehyde showed excellent activity against vesicular stomatitis virus.

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Summary

Two compounds, 4',5'-unsaturated adenosine-2',3'-dialdehyde and tubercidin-2'3'-dialdehyde were submitted during the reporting period. Work on the synthesis of adenosine-2'-monoaldehyde, adenosine-3'-monoaldehyde and 5'-deoxyadenosine-2',3'-dialdehyde is still in progress. As a group the nucleoside dialdehydes thus far submitted have shown broad spectrum activity against many of the viruses in the screening system, and some, like guanosine dialdehyde, have shown remarkably low cytotoxicity. Even dialdehydes that cannot become phosphorated, which is required for the antiviral activity of all clinically used nucleoside antivirals, showed antiviral activity. For example 5'-fluoro-5'-deoxyadenosine-2',3'-dialdehyde showed good activity against yellow fever and 4',5'-unsaturated adenosine-2',3'-dialdehyde showed excellent activity against vesic lar stomatitis virus.

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Foreword

Citations of commerical organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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Technical Presentation

Background

Last contract year, compounds 1-5,7 and 9 were prepared by periodic acid oxidation of: inosine, 6-methylmercaptopurine riboside, thymine riboside, guanosine, 5'-fluoro-5'-deoxyadenosine (compound 6), 8-bromo-adenosine and N^6, N^6-dimethylaminopurine riboside, respectively. The synthesis of these compounds, which were submitted for antiviral screening during the last contract year, were described in the last annual report. Work toward the synthesis of the 4',5'-unsaturated derivative (8) of adenosine dialdehyde, as well as the unsuccessful attempt to synthesize the dialdehyde derivative (10) of formycin A was also described in the last annual report, which contains the structures of compounds 1-10.

This annual report describes the successful completion of the synthesis of compound 8 as well as the synthesis of tubercidin-2',3'-dialdehyde (11). Compound 8 and 11 were the only two compounds submitted during this reporting period. This report also describes work in progress toward the synthesis of adenosine-2'-monoaldehyde, adenosine-3'-monoaldehyde, and additional 5'-deoxyadenosine-2',3'-dialdehyde (AVS #1214). Biological results obtained on all compounds thus far submitted for screening is also presented and evaluated in this report.

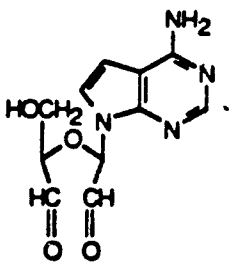
Chemistry

4',5'-Unsaturated adenosine-2',3'-dialdehyde (8) was prepared by modification of the method of Grant and Lerner[1]. Periodate oxidation of tubercidin by the method of Dvonch et al.[2] gave tubercidin-2',3'-dialdehyde (11). Details on the synthesis and characterization of compounds 8 and 11 (Table I) is presented in the Experimental Section.

Work towards the synthesis of adenosine monoaldehydes is described in Scheme I. Periodate oxidation of 5'-O-trityladenosine (Sigma Chemical Co.), compound 12 gave dialdehyde 13, which was then reduced to compound 14 with sodium trimethoxyborohydride. Adduct 14 was treated with excess trityl chloride in an attempt to prepare the ditrityl derivatives 15 and 16. This reaction, however, produced the tritritylated derivative 17 instead of the desired intermediates. The structure of 17 was positively identified by NMR. We are confident that more controlled tritylation will afford the desired 15 and 16, and this reaction is currently underway. Pfitzner-Moffatt oxidation of 15 and 16 should afford blocked monoaldehydes 18 and 19 which should readily be able to be deblocked to afford target monoaldehydes 20 and 21.

Work towards preparation of more AVS #1214 is described in Scheme II. Treatment of adenosine (22) with thionyl chloride in acetonitrile followed by aqueous sodium carbonate hydrolysis of the sulfite intermediate (not shown) has thus far afforded approx. 5 grams of 5'-chloro-5'-deoxyadenosine (23) and work is in progress to prepare still more of this derivative which will be dechlorinated to 24, which in turn will be oxidized to target 25.

Table I. Compounds Submitted During reporting Period



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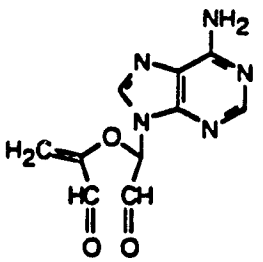

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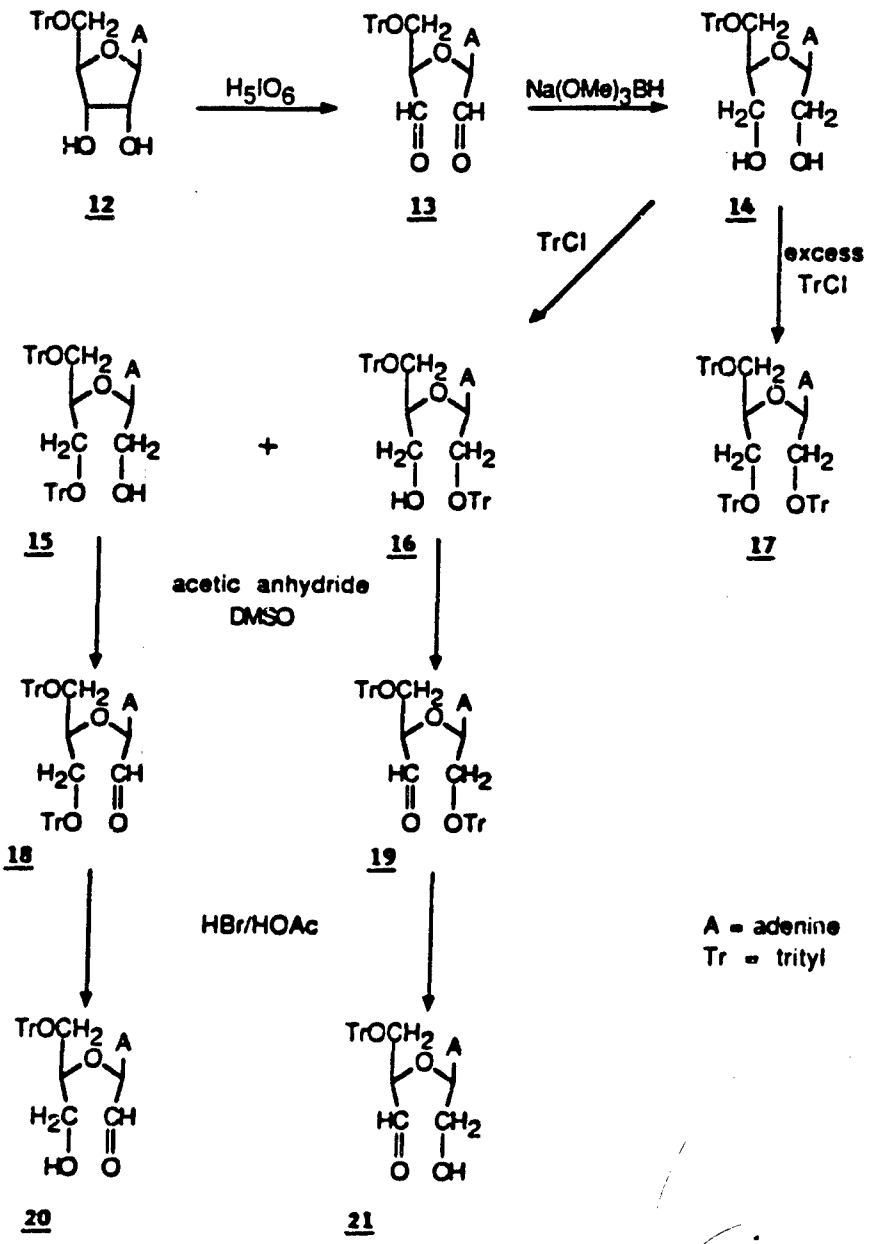
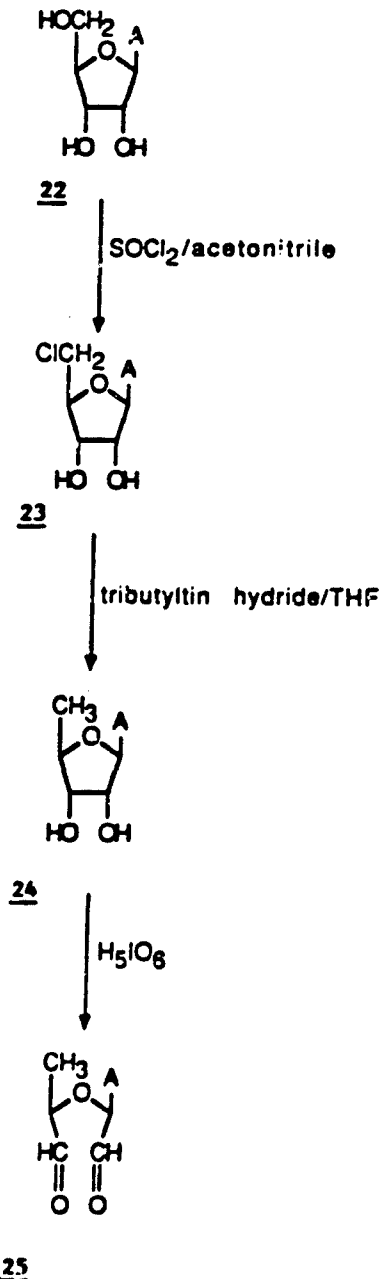
Compound	Code No.	AVS No.	Name
8	OP-I-143	2906	4',5'-Unsaturated adenosine-2',3'-dialdehyde
11	MTN-I-7	2907	Tubercidin-2',3'-dialdehyde

Scheme I


TrOCH2 A
HO OH
12
H5IO6
HC CH
O O
13
Na(OMe)3BH
H2C CH2
HO OH
14
TrCl
excess TrCl
TrO OH
15
+
HO OTr
16
TrO OTr
17
acetic anhydride
DMSO
H2C CH
TrO O
18
HC CH2
O OTr
19
HBr/HOAc
HO O
20
O OH
21
A = adenine
Tr = trityl

Scheme II


HOCH2
O
A
HO OH
22
SOCl2/acetonitrile
ClCH2
O
A
HO OH
23
tributyltin hydride/THF
CH3
O
A
HO OH
24
H5IO6
CH3
O
A
HC CH
O O
25

Biological Results.

Antiviral screening data on all compounds submitted during the contract period are presented in Tables II-XI. Salient results include the following encouraging findings.

1. Compound 8, which cannot become phosphorylated intracellulary, showed a 3.5-fold increase in therapeutic index and a 30-fold decrease in cytotoxicity against vesicular stomatitis virus (VSV) than adenosine dialdehyde (AVS #1160), which, at least theoretically, can become phosphorylated, and which previously was the most active compound against VSV in the entire screening system.

2. Inosine dialdehyde (1), which was virtually inactive in vitro, did show in vivo activity against Crimean-Congo hemorrhagic fever in mice.

3. 5'-Fluoro-5'-deoxyadenosine dialdehyde (5) showed good activity against yellow fever while the parent nucleoside 6 did not. This indicates that the dialdehyde groups are crucial to the antiviral activity of compound 5. Thymine riboside dialdehyde 3 was also active against yellow fever.

4. In one test, compound 7 showed significant activity against the AIDS virus (HIV), but this result will have to be confirmed.

5. As a class the nucleoside dialdehydes thus far submitted both prior to and during the contract period have shown broad spectrum activity against most of the viruses in the screening system, and some, like guanosine dialdehyde (4) showed remarkably low cytotoxicity.

Conclusions and recommendations.

Plans and recommendations for the current third contract year include the following:

1. Synthetic work described in Scheme I and II is still in progress.

2. 5'-Deoxyadenosine dialdehyde (25) will be converted to monoaldehydes following a synthetic similar to Scheme I.

3. Another attempt will be made to synthesize formycin A dialdehyde (10).

4. Work will commence on efforts to prepare 5'-amino-5'-deoxyadenosine dialdehyde and other remaining target compounds listed in the contract proposal.

5. Thymine riboside dialdehyde (1) is somewhat similar in structure to AZT, was active against yellow fever and should be screened against HIV as soon as possible.

6. Other future target compounds will be described in a forthcoming contract renewal proposal.

Table II. Antiviral Activity of Inosine-2',3'-dialdehyde (Compound 1, AVS # 1915)

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+
PT	0.4		220.00	MK2	32.00	0.1	
VSV			NOT ACT	VERO	<250.00	NOT ACT	
VEE			NOT ACT	VERO	<250.00	NOT ACT	
RVF			NOT ACT	VERO	<250.00	NOT ACT	
YF			NOT ACT	MK2	<250.00	NOT ACT	
PIC			NOT ACT	VERO	< 50.00	NOT ACT	
PIC			NOT ACT	VERO	<250.00	NOT ACT	
VSV	0.0	1.0	NOT ACT	L929	100.00	0.0	3.4
AD2	>0.2	0.5	31.95	HEP2	<100.00	<3.1	2.4
VV	0.2	0.8	320.00	VERO	320.00	1.0	2.8
PT	>0.0	0.5	NOT ACT	VERO	>320.00	>0.0	4.6
JBE	>0.0	0.9	NOT ACT	VERO	>320.00	>0.0	2.2
SFS	0.3	0.7	26.19	VERO	32.00	1.2	4.6
YF	0.1	0.8	NOT ACT	VERO	100.00	0.0	1.4
PT	0.0	0.4	NOT ACT	VERO	320.00	0.0	4.6
HIV	0.0	2.2	NOT ACT	ATH8	32.00	0.0	>320.0

IN VIVO SCREEN [mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	RTE	D
cch	mus	2.0	2.4	50 mg	>50	IP	1
cch	mus	0.8	2.7	50 mg	>50	IP	1

Table III. Antiviral Activity of 6-Methylmercaptopurine Riboside-2',3'-dialdehyde (Compound 2, AVS #1970)

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+
PT	0.1		24.00	MK2	3.20	0.1	
RVF			NOT ACT	VERO	25.00	NOT ACT	
VSV	0.2	1.0	17.88	L929	32.00	1.8	3.4
AD2	>0.1	0.5	NOT ACT	HEP2	<10.00	0.0	2.4
VV	0.0	0.8	NOT ACT	VERO	32.00	0.0	2.8
PT	>0.0	0.5	NOT ACT	VERO	>320.00	0.0	4.6
JBE	0.1	0.8	32.00	VERO	32.00	1.0	1.1
YF	0.0	0.6	NOT ACT	VERO	>320.00	0.0	1.8
SFS	>0.0	0.8	NOT ACT	VERO	>320.00	0.0	8.5
PT	0.0	0.4	NOT ACT	VERO	>320.00	>0.0	4.6

Table IV. Antiviral Activity of Thymine Riboside-2',3'-dialdehyde (Compound 3, AVS #1976)

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+
VSV	0.6		100.00	L929	100.00	1.0	
RVF			NOT ACT	VERO	50.00	NOT ACT	
AD2	0.2		NOT ACT	HEP2	32.00	NOT ACT	
SF	>0.8		16.43	VERO	>100.00	>6.1	
PIC			67.00	VERO	>100.00	>1.5	
YF	0.9		18.05	VERO	320.00	17.7	
JE	>0.3		70.92	VERO	>100.00	>1.4	
PT	0.6		45.00	MK2	3.20	0.1	
PT	0.7		25.00	MK2	1.00	0.0	
PTVB	0.4		85.00	MK2	10.00	0.1	
YF	0.1	0.6	NOT ACT	VERO	3.20	0.0	0.2
PT	0.0	0.4	NOT ACT	VERO	>320.00	>0.0	4.6
VEE	0.0	0.3	NOT ACT	VERO	100.00	0.9	1.2

Table V. Antiviral Activity of Guanosine-2',3'-dialdehyde (Compound 4, AVS #1211)

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+
RVF			NOT ACT	VERO	>562.40	NOT ACT	
VEE			NOT ACT	VERO	>562.40	NOT ACT	
YF			<562.40	MK2	>562.40	>1.0	
VSV			73.43	VERO	>562.40	>7.7	
PIC			NOT ACT	VERO	>562.40	NOT ACT	
PIC			NOT ACT	VERO	568.00	NOT ACT	
RVF			NOT ACT	VERO	568.00	NOT ACT	
VEE			NOT ACT	VERO	568.00	NOT ACT	
VSV			0.26	VERO	568.00	7.7	
YF			2.00	MK2	568.00	1.0	
AD2	>0.4	0.8	4.91	HEP2	<32.00	<6.5	2.7
VV	0.5	1.0	62.11	VERO	320.00	5.2	2.8
PT	>0.0	0.5	NOT ACT	VERO	>320.00	>0.0	4.6
YF	0.0	0.7	NOT ACT	VERO	32.00	0.0	0.3
SFS	0.4	1.0	13.27	VERO	32.00	2.4	18.6
JBE	>0.1	1.0	NOT ACT	VERO	320.00	>0.0	6.4
PT	0.1	0.4	253.49	VERO	320.00	1.3	1.1

Table VI. Antiviral Activity of 5'-Fluoro-5'deoxyadenosine-2',3'-dialdehyde (Compound 5, AVS #2275)

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+
RVF			NOT ACT	VERO	<250.00	NOT ACT	
AD2	0.5		57.50	HEP2	100.00	1.7	
VV	0.7		14.81	VERO	100.00	6.8	
VSV	0.1		31.00	L929	10.00	0.3	
SF	0.7		13.55	VERO	100.00	7.4	
FeLV	0.1		NOT ACT	81C	10.00	0.0	
YF	1.1		13.13	VERO	320.00	24.4	
HIV	0.3	1.4	NOT ACT	ATH8	10.00	0.0	>32.0
JE	0.2		61.68	VERO	100.00	1.6	
HIV	0.0		NOT ACT	ATH8	32.00	0.0	
YF	0.2	0.6	79.56	VERO	10.00	0.1	0.2

Table VII. Antiviral Activity of 5'-Fluoro-5'deoxyadenosine (Compound 6, AVS #2273).

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+
VSV	0.0		NOT ACT	L929	100.00	0.0	
AD2	>0.1		NOT ACT	HEP2	<100.00	0.0	
VV	0.1		NOT ACT	VERO	100.00	0.0	
YF	0.3		98.43	VERO	10.00	0.1	
SF	0.0		NOT ACT	VERO	100.00	0.0	
JE	0.0		NOT ACT	VERO	10.00	0.0	

Table VIII. Antiviral Activity of 8-Bromoadenosine-2',3'-dialdehyde (Compound 7, AVS #2274)

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+
RVF			132.47	VERO	<250.00	1.90	
AD2	>0.1		NOT ACT	HEP2	<100.00	0.0	
VV	0.1		NOT ACT	VERO	32.00	0.0	
VSV	0.1		NOT ACT	L929	32.00	0.0	
SF	0.7		15.88	VERO	100.00	6.3	
YF	0.4		22.35	VERO	3.20	0.1	
FeLV	0.0		NOT ACT	81C	10.00	0.0	
HIV	0.4	1.2	0.32	ATH8	3.20	10.0	>32.0
JE	0.3		91.75	VERO	100.00	1.1	
HIV	>0.0		NOT ACT	ATH8	<100.00	<0.0	

Table IX. Antiviral Activity of 4',5'-Unsaturated Adenosine-2',3-dialdehyde (Compound 8, AVS #2906)

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+
VSV	>0.7	1.3	7.81	L929	<100.00	<12.8	5.9
VSV	0.9	1.0	8.86	L929	100.00	11.3	3.4
AD2	0.2	0.7	57.02	HEP2	32.00	0.6	4.8
VV	0.7	0.9	11.45	VERO	100.00	8.7	2.6
JBE	0.0	0.7	NOT ACT	VERO	100.00	0.0	2.0
JBE	0.0	0.7	NOT ACT	VERO	100.00	0.0	2.0
YF	0.8	0.7	24.68	VERO	320.00	13.0	0.2
SFS	0.7	0.9	24.82	VERO	100.00	4.0	14.0
RVF			335.83	VERO	>250.00	0.7	3.7
HIV	0.0	2.1	NOT ACT	ATH8	10.00	0.0	>320.00

Table X. Antiviral Activity of N^6,N^6-Dimethylaminopurine Riboside-2',3'-dialdehyde (Compound 9, AVS #2543)

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+
AD2	>0.1		NOT ACT	HEP2	<32.00	0.0	
VSV	0.1		31.01	L929	32.00	1.0	
VV	0.0		NOT ACT	VERO	32.00	0.0	
JE	0.0		NOT ACT	VERO	32.00	0.0	
PT	0.3	1.0	59.82	VERO	100.00	1.7	10.8
YF	0.0	0.8	NOT ACT	VERO	32.00	0.0	1.9
SF	0.5	1.1	10.34	VERO	32.00	3.1	13.8
HIV	0.5	1.4	NOT ACT	ATH8	32.00	0.0	>320.0
PT	0.7		30.00	MK2	3.20	0.1	3.5

Table XI. Antiviral Activity Tubercin-2'3'-dialdehyde (Compound 11, AVS #2907)

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+
RVF			206.00	VERO	>250.00	1.2	>3.7
VSV	0.1	1.3	23.92	L929	32.00	1.3	5.9
AD2	0.3	0.7	17.77	HEP2	32.00	1.8	4.8
VV	0.0	0.9	NOT ACT	VERO	100.00	0.0	2.6
JBE	0.0	0.7	NOT ACT	VERO	100.00	0.0	2.0
SFS	0.9	0.9	16.40	VERO	320.00	19.5	14.0
YF	0.3	0.7	199.91	VERO	320.00	1.6	0.2

Legend to Tables II-XI

IN VITRO SCREEN - In vitro test results

VIR Symbol	Virus
VEE	Venezuelan Equine Encephalomyelitis
YF	Yellow Fever
JBE	Japanese Encephalitis Virus
PIC	Pichinde
RVF	Rift Valley Fever
SFS	Sandfly Fever (Sicilian)
PT	Punta Toro Virus
CCH	Crimean-Congo Hemorrhagic Fever
VSV	Vesicular Stomatitis Virus
AD2	Adenovirus Type 2
VV	Vaccinia
FeLV	Feline Leukemia Virus
HIV	Human Immunodeficiency Virus

VR	Virus rating calculated by the method of Hoffman and Sidwell (Appl. Microbiol. 22: 795-801, 1971).
VR+	Virus rating for positive control.
ID50	Inhibitory dose 50. Concentration of the drug that causes a 50% reduction in virus replication.
CELL	Cell Line.
MTC	Minimum toxic concentration. The lowest concentration of the test compound that results in a 50% reduction in the percent survival of viable host cells.
TI	Therapeutic index. A measure of the antiviral potential of a compound calculated as MTC/ID50.
TI+	Therapeutic index of positive controls.

IN VIVO SCREEN - In vivo test results

VIR	Virus (see column 2)
HST	Host: MUS = Mouse
VR	Virus rating--a measure of the in vivo antiviral potential of the drug. Calculated as:

$$\frac{\text{GEOMETRIC MTD OF EXPERIMENTAL GROUP}}{\text{GEOMETRIC MTD OF PLACEBO GROUP}}$$

VR+	VR for postive control.
DOSE	Milligrams per kilogram of body weight
MTC	Maximum tolerated dose
RTE	Route of drug administration: IP-intraperitoneal
D	Schedule of administration: 1 - Single dose on day 1

Experimental Section

IR spectra were recorded on a Perkin Elmer 681 Infrared Spectrophotometer. ^1H NMR spectra were recorded on an IBM WP 200 SY instrument. Chemical shift values are reported in δ relative to Me_4Si. UV spectra were recorded on a Varian Cary 219 Spectrophotometer. Mass spectra were obtained in the electron impact mode using a direct insertion probe and recorded on a Hewlett Packard 5995 Gas Chromatograph/Mass Spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

4',5'-Unsaturated adenosine-2',3'-dialdehyde, 8 (AVS #2906).

Adenosine-2',3'-dialdehyde[2] (3.0 g, 11.3 mmol) in 200 mL of water was heated under reflux for one hr under a positive pressure of N_2. The crude reaction mixture, containing approx 50% of 8, as indicated by analytical HPLC (column: 4 mm x 30 cm C_{18} column; mobile phase: 5 vol% acetonitrile in water) was partially purified on a Waters Prep LC 500A system (column: 57 mm x 30 cm C_{18} cartridge; mobile phase: 3 vol% acetonitrile in water; flow rate: 200 mL/min; detector: refractive index). Solvents were removed by rotary evaporation under high vacuum to a small volume, followed by lyophilization to give 8 in 85% purity. The above procedure was repeated twice. The product of all three reactions was combined, dissolved in a minimum amount of hot water (approx 175 mL), and further purified on the Prep LC 500A system as described above. Solvents were removed as described above to give 1.5 g (17% yield) of 95% pure 8, as indicated by analytical HPLC.

Physical and Analytical Data

Melting Point: 196° (dec)

Analysis: For $C_{10}H_9N_5O_3.1.18H_2O$ (268.47)

	Calcd	Found
C	44.74	44.63
H	4.26	3.86
N	26.08	25.72

IR Spectrum: KBr. Compatible with structure. Broad band at 1100 cm^{-1} typical of nucleoside dialdehydes.[3]

NMR (D_2O):

δ 9.21 (s, 1 H, H-3'), 8.28 (s,1 H, H-8), 8.18 (s, 1 H, H-2), 5.99-5.97 (d, 1 H, H-1', $J_{H-1', H-2'}$ = 5.2 Hz), 5.77-5.76 (d, 1 H, H-5', $J_{H-5', H-5'}$ = 3.2 Hz), 5.46-5.44 (d, 1 H, H-2', $J_{H-2', H-1'}$ = 5.2 Hz), 5.41-5.40(d, 1 H, H-5', $J_{H-5', H-5'}$ = 3.2 Hz).

UV Spectrum: λ_{max} (H_2O) 256 nm (ε 15,957)

Mass Spectrum (EI):

m/z 247 (M^+), 218 (M^+ - CHO).

Thin-layer Chromatography: Eastman 13254 cellulose

Eluent	Rf	Comment
H_2O	0.68	Homogeneous
EtOH-1 M NH_4OAc (7:3)	0.64	Homogeneous

Code No.: OP-I-143

Prepared by: S. M. Opitz

Tubercidin-2',3'-dialdehyde, 11 (AVS #2907).

Prepared by the general method of Dvonch et al[2]. To a stirred suspension of 2.96 g (11.12 mmol) of tubercidin in 120 mL of water was added 2.94 g (12.23 mmol) of periodic acid. The reaction mixture was stirred for one hr at room temp and then applied to a 1.5 x 20.0 cm column of Bio-Rad AG 1-X8 anion exchange resin (acetate form). The column was washed with water. The self-eluate and washings (400 mL) were found to be free of iodate and periodate by starch iodide test paper, and were lyophilized to give 2.31 g (68% yield) of 11.

Physical and Analytical Data

Melting Point: >132° (dec)

Analysis: For $C_{11}H_{12}N_4O_4.1.6H_2O$

	Calcd	Found
C	45.08	44.95
H	5.23	5.31
N	19.12	18.98

IR Spectrum: KBr. Broad band at 1100 cm^{-1} typical of nucleoside dialdehydes.[3]

NMR (D_2O):

δ 8.17-8.00 (m, 1 H, H-2), 7.97-5.91 (m, 2 H, H-7, H-8), 5.87-4.88 (m, 3 H, H-1', H-2', H-3'), 4.50-3.36 (m, 3 H, H-4', 2 H-5').

UV Spectrum: λ_{max} (H_2O) 268 nm (ε 11,500).

Mass Spectrum (EI):

m/z 264 (M^+), 282 ($M^+ + H_2O$)

Thin-layer Chromatography: Eastman 13254 cellulose

Eluent	RF
H_2O	0.61
EtOH-1 M NH_4OAc	0.81

Code No.: MTN-I-7

Prepared by: M. T. Nemergut

Materials:

Tubercidin	Sigma Chem Co. Lot #106F-7080
Periodic acid	Sigma Chem Co. Lot #34F-0351
Anion exchange resin (acetate form)	Bio-Rad AG 1-X8 Control #28817

Literature Cited

1. A. J. Grant and L. M. Lerner, J. Med. Chem., 23, 795 (1980).

2. W. Dvonch, H. Fletcher, III, F. J. Gregory, E-M. H. Healy, G. H. Warren, H. E. Alburn, Cancer Res., 26, 2386 (1966).

3. F. Hanseke and F. Cramer, Carbohydr. Res., 54, 75 (1977).

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